AMENDMENTS TO THE SPECIFICATION

Please amend the follow paragraph of page 15 to read as follows:

(c) a polynucleotide that encodes a 158P1D7-related protein whose sequence is encoded by the cDNAs contained in the plasmid designated p158P1D7- Turbo/3PX deposited with American Type Culture Collection as Accession No. PTA- ~~[ ]~~ 3662 on 22 August 2001 (sent via Federal Express on 20 August 2001);

Please amend the follow paragraphs of page 8 to read as follows:

Figure 11. Hydrophilicity amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Hopp and Woods (Hopp T.P., Woods K.R., 1981. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828) accessed on the Protscale website ~~(www.expasy.ch/cgi-bin/protscale.pl)~~ (w--.expasy.ch/cgi-bin/protscale.pl) through the ExPasy molecular biology server.

Figure 12. Hydropathicity amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Kyte and Doolittle (Kyte J., Doolittle R.F., 1982. J. Mol. Biol. 157:105-132) accessed on the ProtScale website ~~(www.expasy.ch/cgi-bin/protscale.pl)~~ through the ExPasy molecular biology server.

Figure 13. Percent accessible residues amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Janin (Janin J., 1979 Nature 277:491-492) accessed on the ProtScale website ~~(www.expasy.ch/cgi-bin/protscale.pl)~~ through the ExPasy molecular biology server.

Figure 14. Average flexibility amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Bhaskaran and Ponnuswamy (Bhaskaran R., and Ponnuswamy P.K., 1988. Int. J. Pept. Protein Res. 32:242-255) accessed on the ProtScale website ~~(www.expasy.ch/cgi-bin/protscale.pl)~~ through the ExPasy molecular biology server.

Figure 15. Beta-turn amino acid profile of 158P1D7 determined by computer algorithm sequence analysis using the method of Deleage and Roux (Deleage, G., Roux B. 1987 Protein Engineering 1:289-294) accessed on the ProtScale website ~~(www.expasy.ch/cgi-bin/protscale.pl)~~ through the ExPasy molecular biology server.

Please amend the follow paragraph of page 21 to read as follows:

As discussed herein, redundancy in the genetic code permits variation in 158P1D7 gene sequences. In particular, it is known in the art that specific host species often have specific codon preferences, and thus one can adapt the disclosed sequence as preferred for a desired host. For example, preferred analog codon sequences typically have rare codons (i.e., codons having a usage frequency of less than about 20% in known sequences of the desired host) replaced with higher frequency codons. Codon preferences for a specific species are calculated, for example, by utilizing codon usage tables available on the INTERNET such as at URL ~~www.dna.affrc.go.jp/~nakamura/codon.html~~ w--.dna.affrc.go.jp/~nakamura/codon.html.

Please amend the follow paragraph of page 24 to read as follows:

Additional illustrative embodiments of the invention disclosed herein include 158P1D7 polypeptides comprising the amino acid residues of one or more of the biological motifs contained within the 158P1D7 polypeptide sequence set forth in Figure 2 or Figure 3. Various motifs are known in the art, and a protein can be evaluated for the presence of such motifs by a number of publicly available Internet sites (see, e.g., URL addresses: ~~pfam.wustl.edu/;~~ ~~searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html~~ pfam.wustl.edu/; searchlauncher.bcm.tmc.edu/seq-search/struc-predict.html; ~~psort.ims.u-tokyo.ac.jp/;~~ ~~www.cbs.dtu.dk/; www.ebi.ac.uk/interpro/scan.html;~~ psort.ims.u-tokyo.ac.jp/; w--.cbs.dtu.dk/; w--.ebi.ac.uk/interpro/scan.html; ~~www.expasy.ch/tools/scnpsit1.html;~~ w--.expasy.ch/tools/scnpsit1.html; Epimatrix™ and Epimer™, Brown University, ~~www.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html~~ w--.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html; and BIMAS, ~~bimas.dcrt.nih.gov/.)~~ bimas.dcrt.nih.gov/.

Please amend the follow paragraph of page 25 to read as follows:

In another embodiment, proteins of the invention comprise one or more of the immunoreactive epitopes identified in accordance with art-accepted methods, such as the peptides set forth in Tables V-XVIII. CTL epitopes can be determined using specific algorithms to identify peptides within an 158P1D7 protein that are capable of optimally binding to specified HLA alleles (e.g., Table IV; Epimatrix™ and Epimer™, Brown University, URL ~~www.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html~~ w--.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html; and BIMAS, URL ~~bimas.dcrt.nih.gov/~~ bimas.dcrt.nih.gov.) Moreover, processes for identifying peptides that have sufficient binding affinity for HLA molecules and which are correlated with being immunogenic epitopes, are well known in the art, and are carried out without undue experimentation. In addition, processes for identifying peptides that are immunogenic epitopes, are well known in the art, and are carried out without undue experimentation either *in vitro* or *in vivo*.

Please amend the follow paragraph bridging pages 26-27 to read as follows:

CTL epitopes can be determined using specific algorithms to identify peptides within an 158P1D7 protein that are capable of optimally binding to specified HLA alleles (e.g., by using the SYFPEITHI site at World Wide Web URL syfpeithi.bmi-heidelberg.com/; the listings in Table IV(A)-(E); Epimatrix™ and Epimer™, Brown University, at the URL ~~(www.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html)~~ listed above; and BIMAS, at the URL ~~bimas.dcrt.nih.gov/~~ listed above). Illustrating this, peptide epitopes from 158P1D7 that are presented in the context of human MHC class I molecules HLA-A1, A2, A3, A11, A24, B7 and B35 were predicted (Tables V-XVIII). Specifically, the complete amino acid sequence of the 158P1D7 protein was entered into the HLA Peptide Motif Search algorithm found in the Bioinformatics and Molecular Analysis Section (BIMAS) web site listed above. The HLA peptide motif search algorithm was developed by Dr. Ken Parker based on binding of specific peptide sequences in the groove of HLA Class I molecules, in particular HLA-A2 (see, e.g., Falk et al., Nature 351: 290-6 (1991); Hunt et al., Science 255:1261-3 (1992); Parker et al., J. Immunol. 149:3580-7 (1992); Parker et al., J. Immunol. 152:163-75 (1994)). This algorithm allows location and ranking of 8-mer, 9-mer, and 10-mer

peptides from a complete protein sequence for predicted binding to HLA-A2 as well as numerous other HLA Class I molecules. Many HLA class I binding peptides are 8-, 9-, 10 or 11-mers. For example, for class I HLA-A2, the epitopes preferably contain a leucine (L) or methionine (M) at position 2 and a valine (V) or leucine (L) at the C-terminus (see, e.g., Parker et al., J. Immunol. 149:3580-7 (1992)). Selected results of 158P1D7 predicted binding peptides are shown in Tables V-XVIII herein. In Tables V-XVIII, the top 50 ranking candidates, 9-mers and 10-mers, for each family member are shown along with their location, the amino acid sequence of each specific peptide, and an estimated binding score. The binding score corresponds to the estimated half time of dissociation of complexes containing the peptide at 37°C at pH 6.5. Peptides with the highest binding score are predicted to be the most tightly bound to HLA Class I on the cell surface for the greatest period of time and thus represent the best immunogenic targets for T-cell recognition.

Please amend the follow paragraph of page 44 to read as follows:

CTL epitopes can be determined using specific algorithms to identify peptides within 158P1D7 protein that bind corresponding HLA alleles (see e.g., Table IV; Epimer™ and Epimatrix™, the Brown University ~~[(]~~ URL ~~www.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html)~~ w--.brown.edu/Research/TB-HIV_Lab/epimatrix/epimatrix.html; and, the BIMAS, ~~(URL bimas.dcrt.nih.gov/~~ site listed above; and SYFPEITHI at ~~URL syfpeithi.bmi-heidelberg.com/~~ syfpeithi.bmi-heidelberg.com). In a preferred embodiment, the 158P1D7 immunogen contains one or more amino acid sequences identified using techniques well known in the art, such as the sequences shown in Tables V-XVIII or a peptide of 8, 9, 10 or 11 amino acids specified by an HLA Class I motif/supermotif (e.g., Table IV (A), Table IV (D), or Table IV (E)) and/or a peptide of at least 9 amino acids that comprises an HLA Class II motif/supermotif (e.g., Table IV (B) or Table IV (C)). As is appreciated in the art, the HLA Class I binding groove is essentially closed ended so that peptides of only a particular size range can fit into the groove and be bound, generally HLA Class I epitopes are 8, 9, 10, or 11 amino acids long. In contrast, the HLA Class II binding groove is essentially open ended; therefore a peptide of about 9 or more amino acids can be bound by an HLA Class II molecule. Due to the binding groove differences between HLA Class I and II, HLA Class I motifs are length specific, i.e., position two of a Class I motif is the second amino acid in an amino

to carboxyl direction of the peptide. The amino acid positions in a Class II motif are relative only to each other, not the overall peptide, i.e., additional amino acids can be attached to the amino and/or carboxyl termini of a motif-bearing sequence. HLA Class II epitopes are often 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids long, or longer than 25 amino acids.

Please amend the follow paragraph of page 45 to read as follows:

Vaccine compositions of the invention include nucleic acid-mediated modalities. DNA or RNA that encode protein(s) of the invention can be administered to a patient. Genetic immunization methods can be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing 158P1D7. Constructs comprising DNA encoding a 158P1D7-related protein/immunogen and appropriate regulatory sequences can be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded 158P1D7 protein/immunogen. Alternatively, a vaccine comprises a 158P1D7-related protein. Expression of the 158P1D7-related protein immunogen results in the generation of prophylactic or therapeutic humoral and cellular immunity against cells that bear 158P1D7 protein. Various prophylactic and therapeutic genetic immunization techniques known in the art can be used (for review, see information and references published at Internet address ~~www.genweb.com~~ w--.genweb.com). Nucleic acid-based delivery is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (*see*, *e.g.*, U.S. Patent No. 5,922,687).

Please amend the follow paragraph of page 70 to read as follows:

158P1D7 clone cDNA was deposited under the terms of the Budapest Treaty on 22 August 2001, with the American Type Culture Collection (ATCC; 10801 University Blvd., Manassas, VA

20110-2209 USA) as plasmid p158P1D7- Turbo/3PX, and has been assigned ~~Accession No. [redacted] (docket # [redacted])~~ Patent Deposit Designation PTA-3662.

Please amend the follow paragraph of page 71 to read as follows:

158P1D7 maps to chromosme 13, using 158P1D7 sequence and the NCBI BLAST tool: ~~(http://www.ncbi.nlm.nih.gov/genome/seq/page.cgi?F=HsBlast.html&&ORG=Hs)~~ (w--.ncbi.nlm.nih.gov/genome/seq/page.cgi?F=HsBlast.html&&ORG=Hs). This is a region of frequent amplification in bladder cancer (Prat et al., Urology 2001 May;57(5):986-92; Muscheck et al., Carcinogenesis 2000 Sep;21(9):1721-26) and is associated with rapid tumor cell proliferation in advanced bladder cancer (Tomovska et al., Int J Oncol 2001 Jun;18(6):1239-44).

Please amend the follow paragraph of page 76 to read as follows:

Figure 11, Figure 12, Figure 13, Figure 14, and Figure 15 depict graphically five amino acid profiles of the 158P1D7 amino acid sequence, each assessment available by accessing the ProtScale website ~~(URL www.expasy.ch/cgi-bin/protscale.pl)~~ provided above on the ExPasy molecular biology server.

Please amend the follow paragraph of page 93 to read as follows:

Epitopes are often selected that have a binding affinity of an IC_{50} of 500 nM or less for an HLA class I molecule, or for class II, an IC_{50} of 1000 nM or less; or HLA Class I peptides with high binding scores from the BIMAS web site, provided above ~~at URL bimas.dcrt.nih.gov/~~.

Please amend the follow paragraphs of page 106 to read as follows:

The significant expression of 158P1D7 in cancer tissues, together with its restricted expression in normal tissues, makes 158P1D7 an excellent target for antibody therapy. In cases where the monoclonal antibody target is a cell surface protein, antibodies have been shown to be efficacious at inhibiting tumor growth (See, e.g., (Saffran, D., *et al.*, PNAS 10:1073-~~1078 or www.pnas.org/cgi/doi/10.1073/pnas.051624698~~). In cases where the target is not on the cell

surface, such as PSA and PAP in prostate cancer, antibodies have still been shown to recognize and inhibit growth of cells expressing those proteins (Saffran, D.C., *et al.*, Cancer and Metastasis Reviews, 1999. 18: p. 437-449). As with any cellular protein with a restricted expression profile, 158P1D7 is a target for T cell-based immunotherapy.

Accordingly, the therapeutic efficacy of anti-158P1D7 mAbs in human bladder cancer mouse models is modeled in 158P1D7-expressing bladder cancer xenografts or bladder cancer cell lines, such as those described in Example (the Example entitled "*In Vivo* Assay for 158P1D7 Tumor Growth Promotion", that have been engineered to express 158P1D7.

Antibody efficacy on tumor growth and metastasis formation is confirmed, e.g., in a mouse orthotopic bladder cancer xenograft model. The antibodies can be unconjugated, as discussed in this Example, or can be conjugated to a therapeutic modality, as appreciated in the art. It is confirmed that anti-158P1D7 mAbs inhibit formation of 158P1D7-expressing bladder tumors. Anti-158P1D7 mAbs also retard the growth of established orthotopic tumors and prolong survival of tumor-bearing mice. These results indicate the utility of anti-158P1D7 mAbs in the treatment of local and advanced stages of bladder cancer. (See, e.g., Saffran, D., et al., PNAS 10:1073-1078 ~~or www.pnas.org/cgi/doi/10.1073/pnas.051624698~~).

Please amend the follow paragraph of page 109 to read as follows:

By use of the PubMed website of the N.C.B.I. ~~available at~~ ~~http://www.ncbi.nlm.nih.gov/entrez~~ , it was found at the protein level that 158P1D7 shows best homology to the hypothetical protein FLJ22774 (PubMed record: gi 14149932) of unknown function, with 97% identity and 97% homology. The 158P1D7 protein demonstrates some homology to a human protein similar to IGFALS (insulin-like growth factor binding protein, acid labile subunit) (PubMed record: gi 6691962) with 36% identity and 52% homology and to mouse Slit 1 protein (PubMed record: gi 5532493) with 24% identity and 37% homology (Figures 5a and 5b).

Please amend the follow paragraph of page 110 to read as follows:

Additionally, Figure 16A and 16B set forth a transmembrane region and orientation prediction for 158P1D7. Figure 16A is a schematic representation of the probability of the existence of transmembrane regions and the extracellular and intracellular orientation of 158P1D7 based on the algorithm of Sonnhammer, von Heijne, and Krogh (Erik L.L. Sonnhammer, Gunnar von Heijne, and Anders Krogh: A hidden Markov model for predicting transmembrane helices in protein sequences. In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p 175-182 Ed J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen Menlo Park, CA: AAAI Press, 1998). The method predicts that 158P1D7 contains a single transmembrane region from amino acids 611-633 with high probability that the amino-terminus resides outside, consistent with the topology of a Type 1 transmembrane protein. Also visualized is a short hydrophobic stretch from amino acids 3-25, consistent with the existence of an amino-terminal signal peptide. Figure 16B is a schematic representation of the probability of existence of transmembrane regions and orientation of 158P1D7 based on the TMpred algorithm of Hofmann and Stoffel which utilizes TMBASE (K. Hofmann, W. Stoffel. TMBASE - A database of membrane spanning protein segments Biol. Chem. Hoppe-Seyler 374:166, 1993). The method predicts that 158P1D7 contains a primary transmembrane region from amino acids 609-633 and a secondary transmembrane region from amino acids 3-25 (contiguous amino acids with values greater than 0 on the plot have high probability of being transmembrane regions) with an orientation in which the amino terminus resides inside and the carboxyl terminus outside. An alternative model is also predicted, consistent with Figure 16A, that 158P1D7 is a Type 1 transmembrane protein in which the amino-terminus resides outside and the protein contains a secondary transmembrane domain signal peptide from amino acids 3-25 and a primary transmembrane domain from AA 615-633. The transmembrane prediction algorithms for Figure 16A and Figure 16B are accessed through the ExPasy molecular biology server ~~(http://www.expasy.ch/tools/)~~.

Please amend the follow paragraph of page 1117 to read as follows:

Adapted from the GCG Software 9.0 BLOSUM62 amino acid substitution matrix (block substitution matrix). The higher the value, the more likely a substitution is found in related, natural

proteins. (See URL ~~www.ikp.unibe.ch/manual/blosum62.html~~ w--.ikp.unibe.ch/manual/blosum62.html.)